

BIOIMAGING PLATFORM

Single and multicolor live cell imaging

The confocal SP2 as well as the widefield AF6000 LX microscope are equipped with environmental control boxes and perfusion chambers to optimize conditions for live cells on the microscope stage. In the AF6000 LX microscope we can add a CO₂ and moisture control system.

We will discuss and give advice on media and culture conditions for specific cell types. We will also give advice on fluorescent tags and the use of spectral variants of GFP (green fluorescent protein).

FRET

FRET ("fluorescence resonance energy transfer") is a distance-dependent physical process by which energy is transferred nonradiatively from an excited molecular fluorophore (the donor) to another fluorophore (the acceptor). FRET can therefore be an accurate measurement of molecular proximity at nm distances (1-10 nm) that can be used to obtain spatial and temporal distribution of protein associations in living cells.

The SP2 confocal microscope is especially well suited for FRET measurements. For live cells we currently recommend the use of CPF (cyan fluorescent protein) as the donor fluorophore combined with YFP (yellow fluorescent protein) as the acceptor. Positive and negative (single-transfection) controls are crucial. All that has been mentioned for live cell imaging is of course valid again.

FRAP

The FRAP ("fluorescence recovery after photobleaching") technique is the method of choice to determine the mobility of molecules in living cells. In FRAP, a region of the cell is selectively and intensely irradiated to photobleach fluorescent molecules. The recovery of fluorescent molecules into that region is assessed quantitatively to determine diffusion coefficients and mobile fractions.

The SP2 confocal microscope is extremely well suited to do FRAP measurements. As FRAP is normally done with live cells, incubation conditions on the microscope stage are again critical.